BME

MOLECULAR BIOLOGY EXPERIMENT

DNA CLEANING & ENDONUCLEASE RX

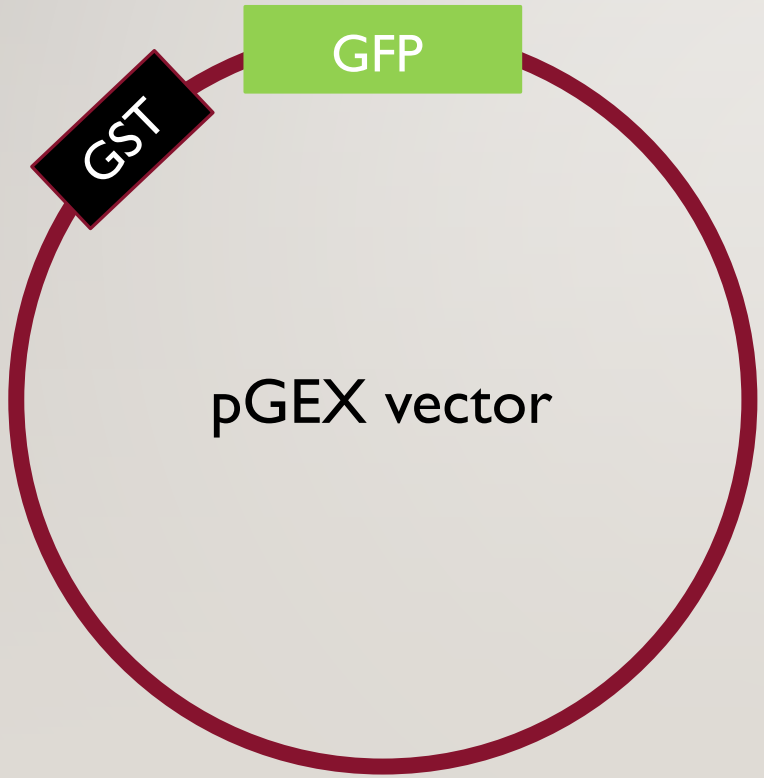
SKKU BME

3RD GRADE, 2ND SEMESTER

TODAY

- Cleaning of PCR product
- Endonuclease reaction of the PCR product for ligation

LET'S SEE THE BIG PICTURE!



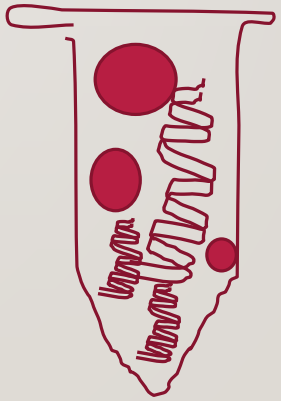
1> Vector
- E.coli transformation & culture
- Miniprep – confirmation by enzyme cutting

2> Insert
- Primer making
- PCR amplification
- Cleaning
- Enzyme cutting for ligation

DNA CLEANING

- Why is it necessary?

- **Think about the PCR product. What did you put in the tube?**



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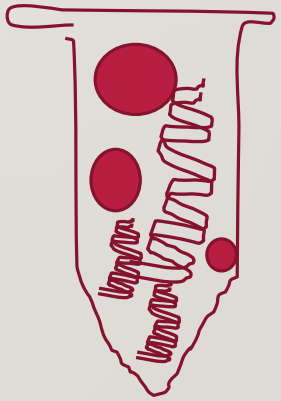

DNA CLEANING

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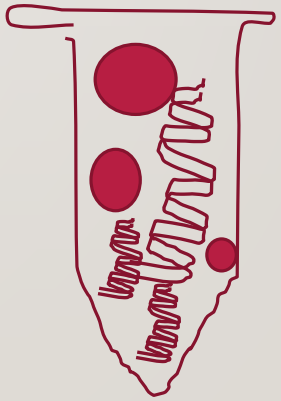
Hint> How to separate DNAs or proteins?



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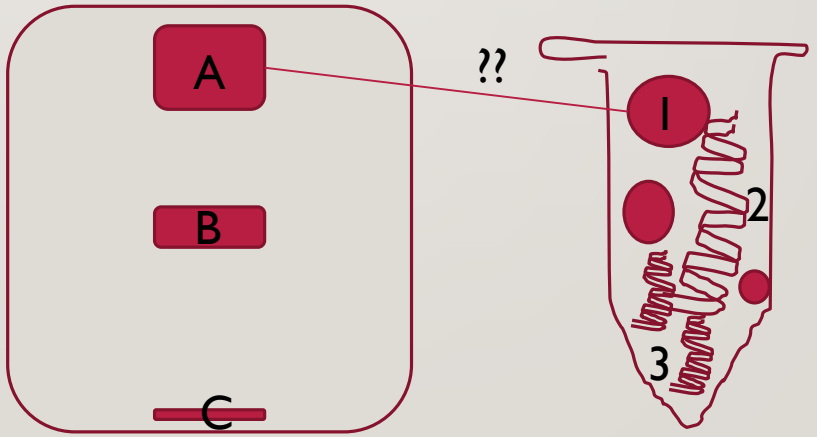
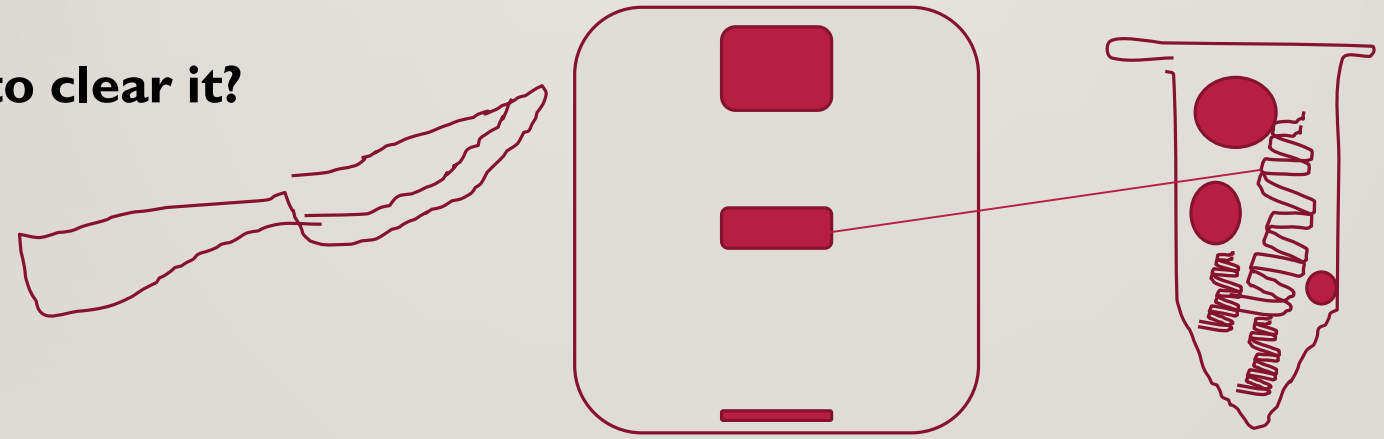


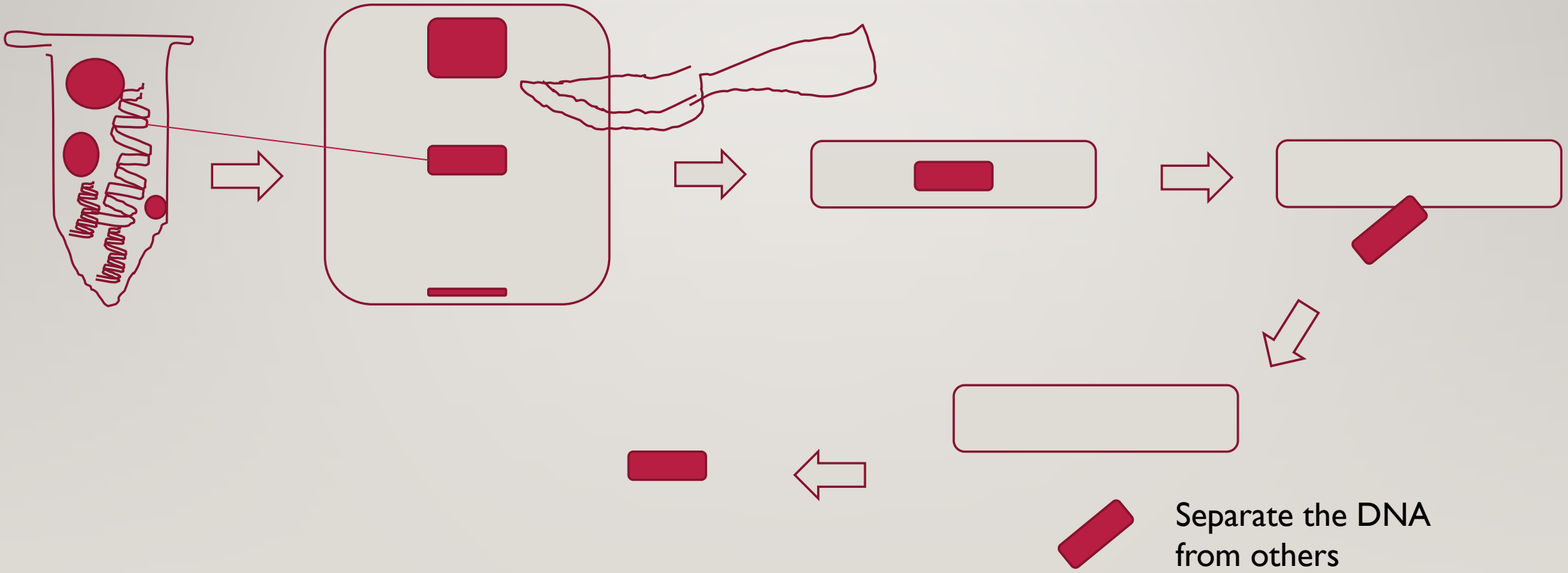
Connect lines

DNA CLEANING

- Why is it necessary?
- **Think about the PCR product. What did you put in the tube?**
- **How to clear it?**



GEL ELUTION METHOD

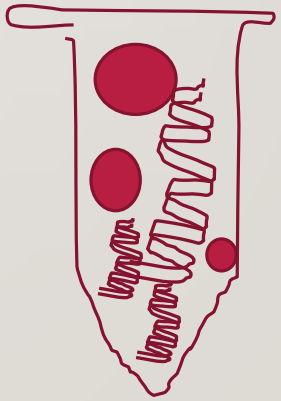


DNA CLEANING – COLUMN METHOD

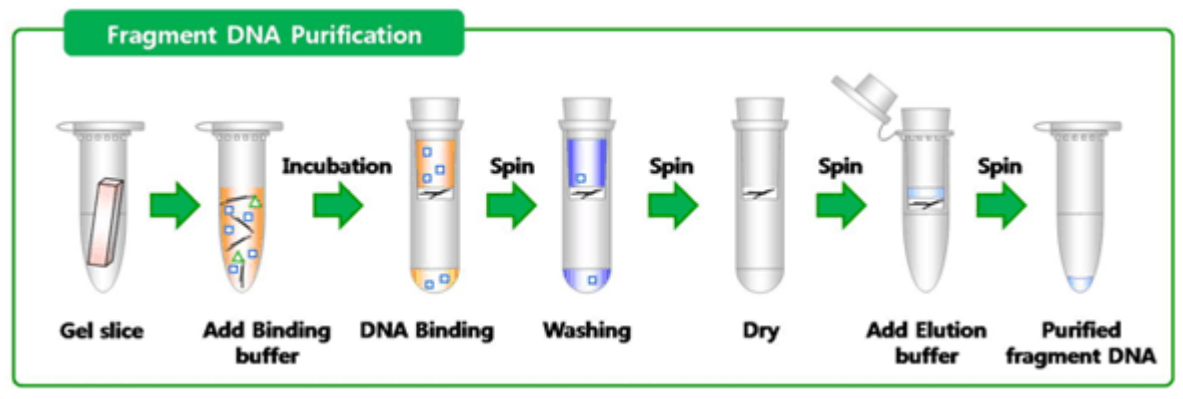
- Why is it necessary?
- **Think about the PCR product. What did you put in the tube?**
- **How to clear it?**

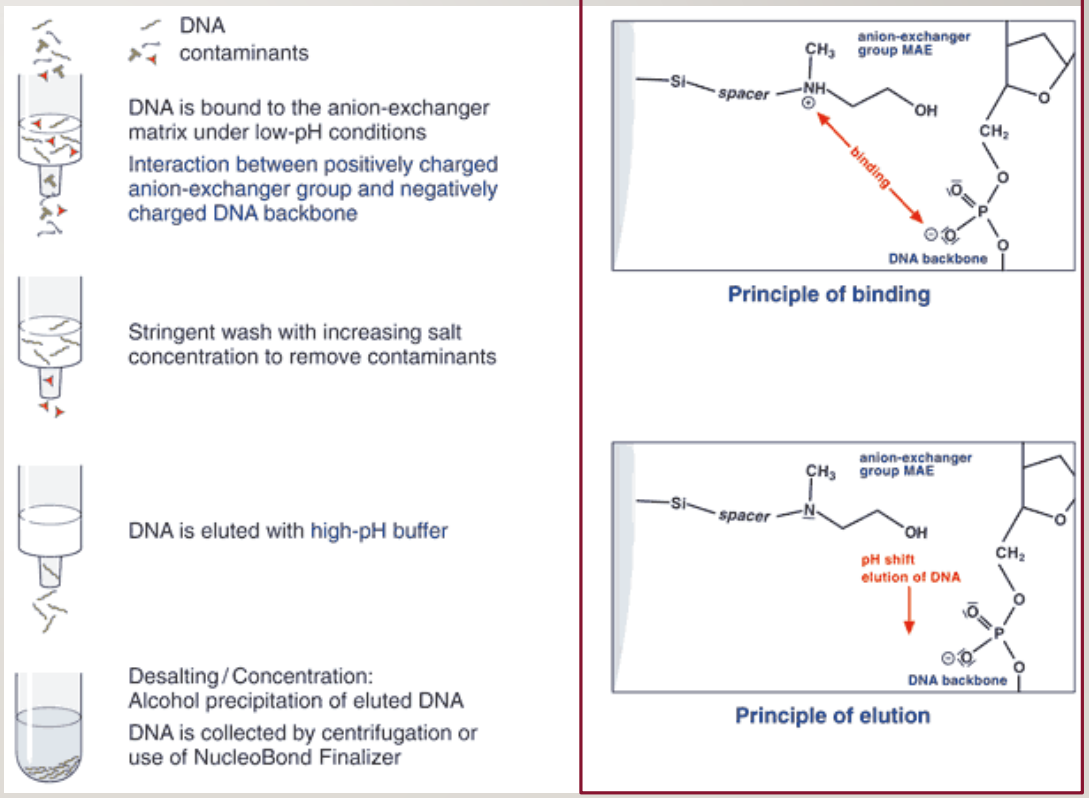
Gel elution method

Column method



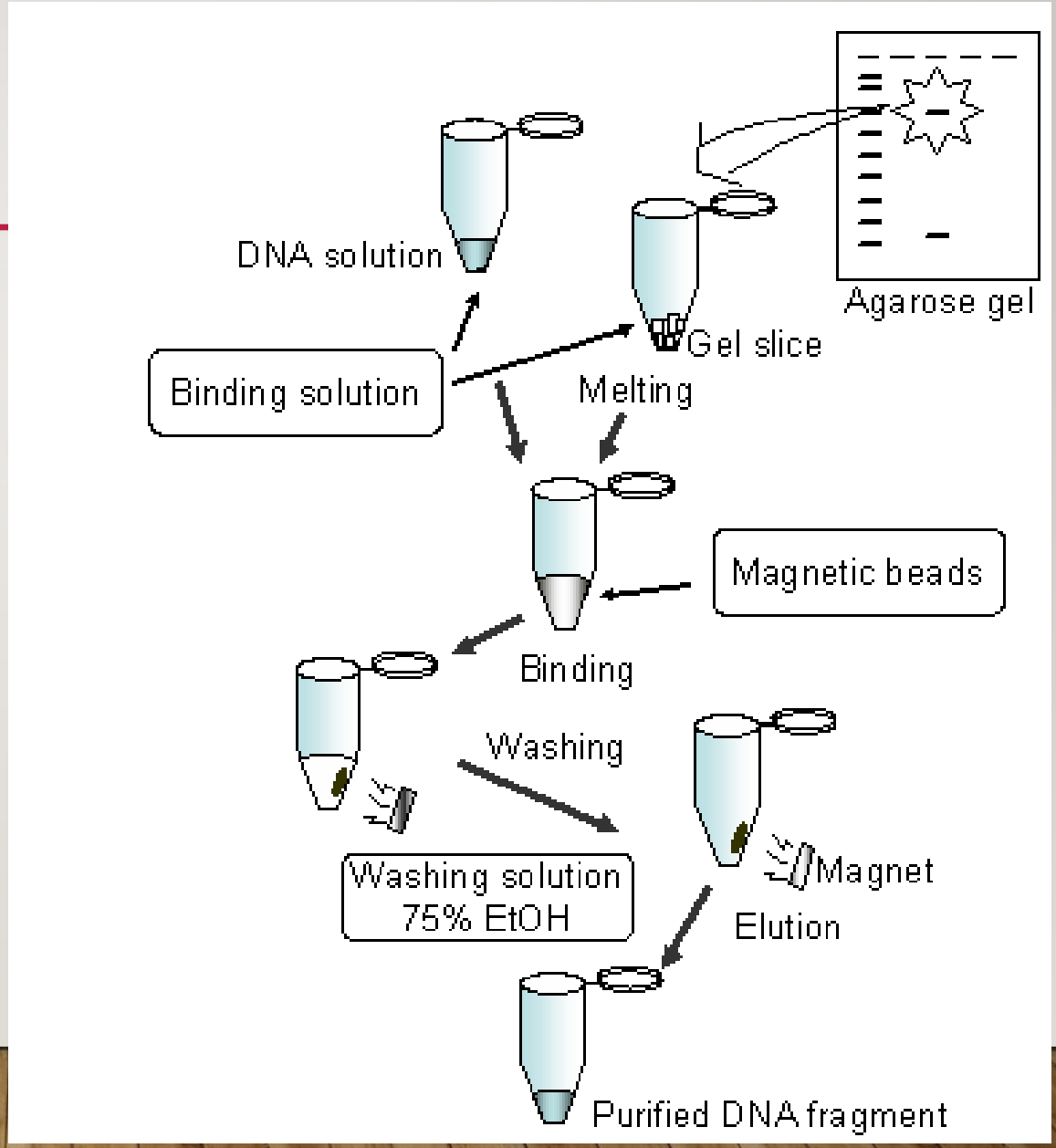
HOW TO SEPARATE THE DNA FROM OTHERS?



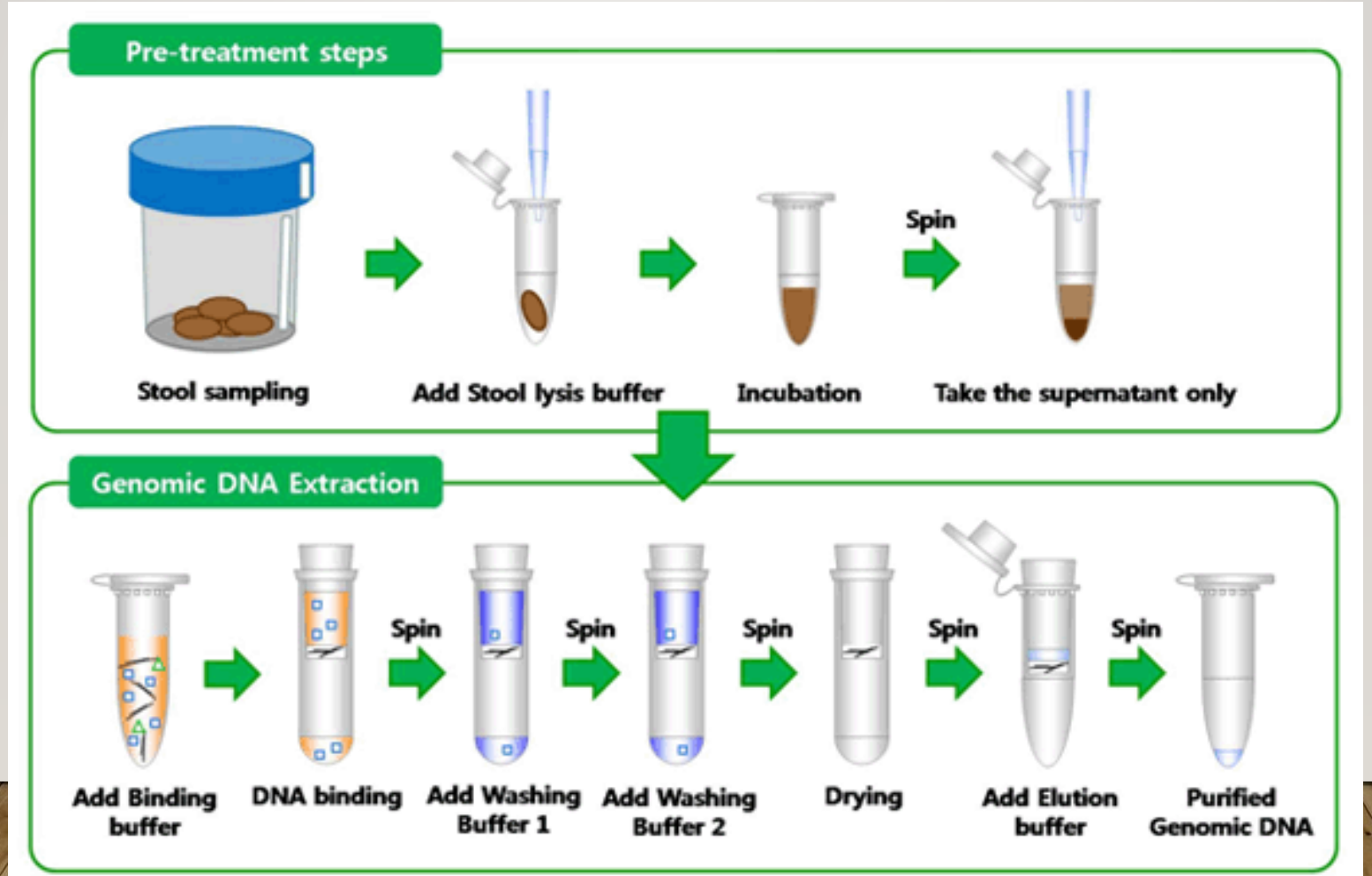


CF) OTHER METHODS…

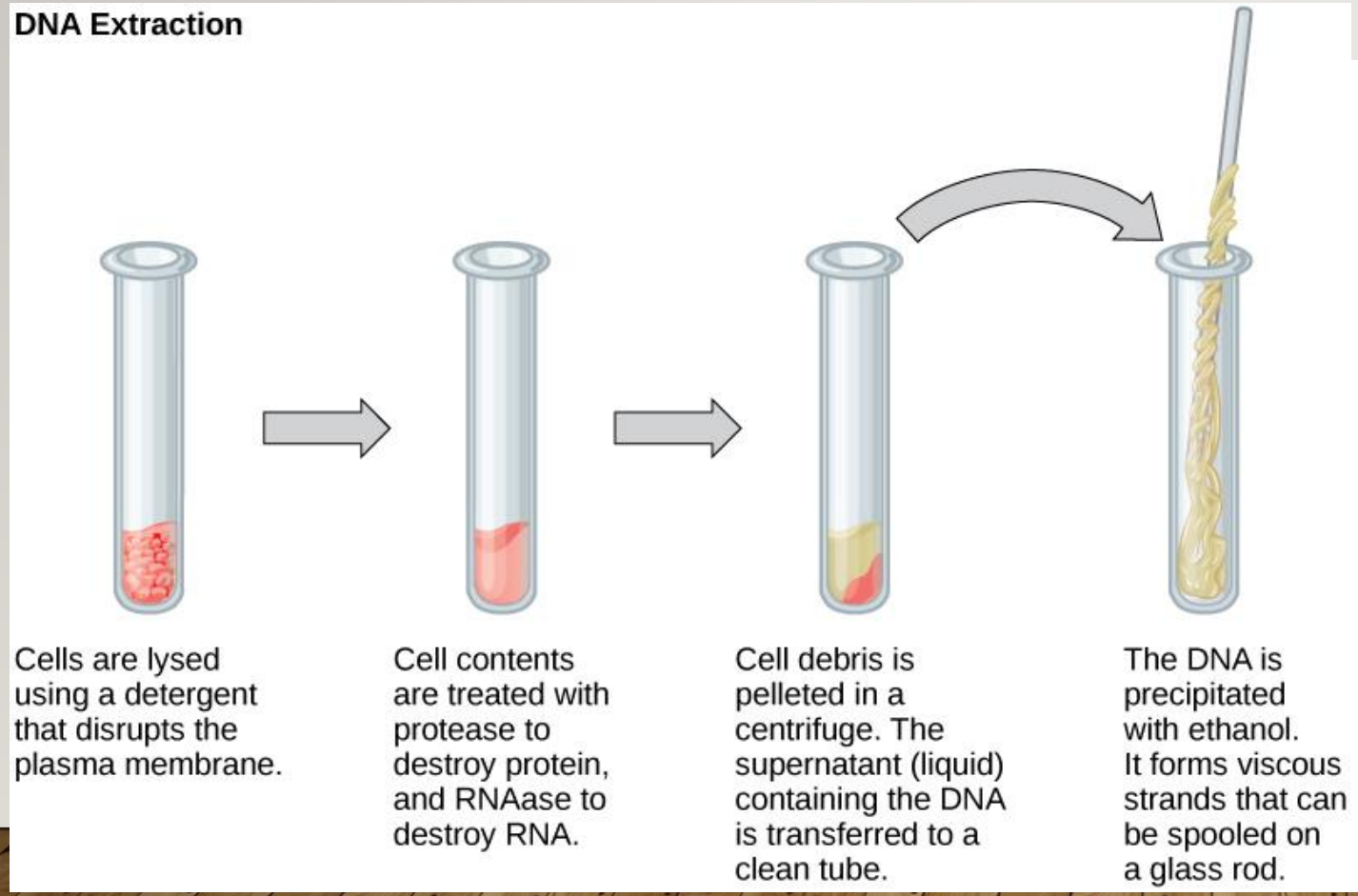
EX) MAGNETIC BEAD



MANY APPLICATIONS…

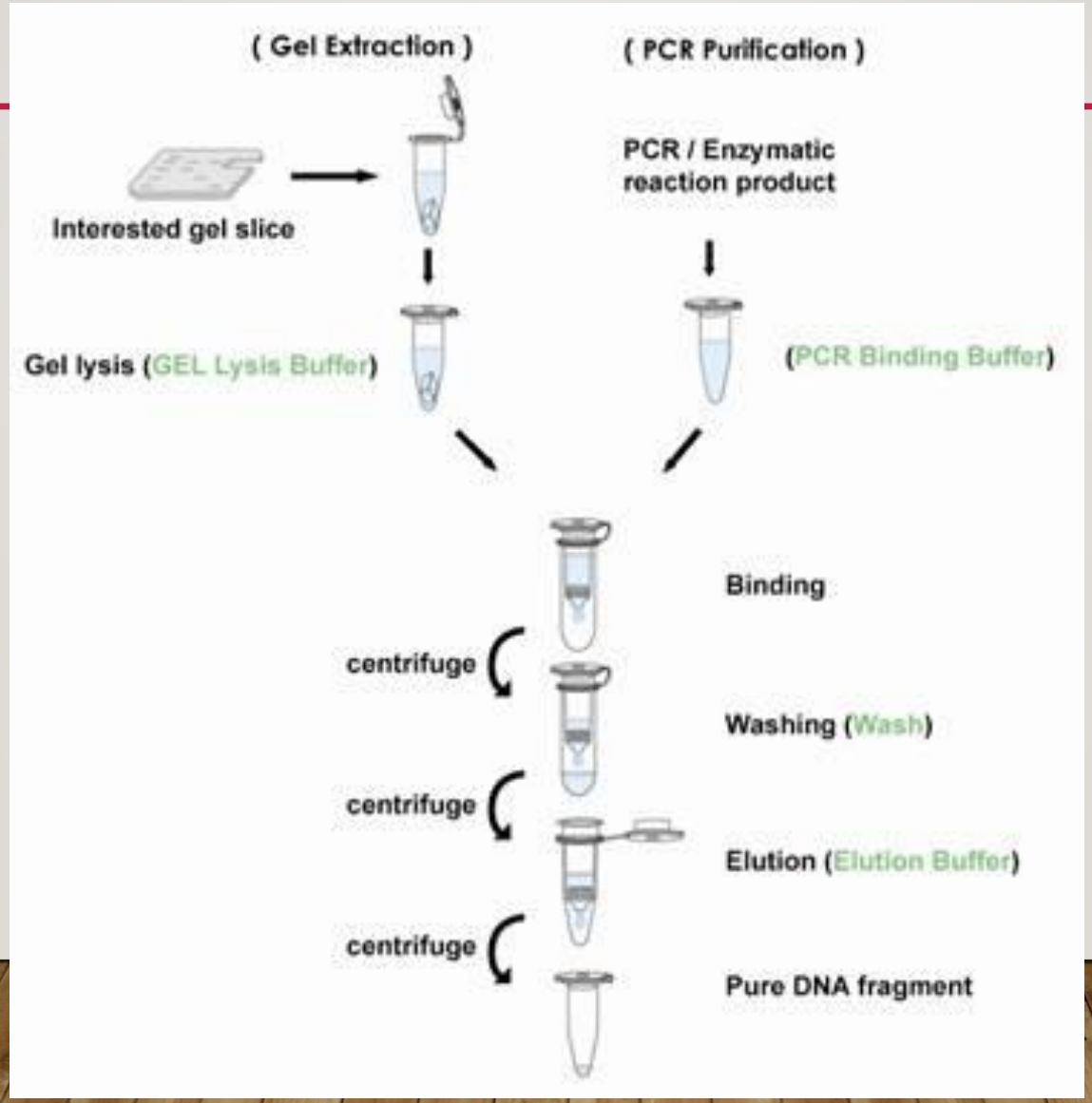


GENOMIC DNA EXTRACTION



- If there is DNA present in any of the samples it should precipitate out in gray clumps that may look like white fine lint fibers.
- Use a **glass rod** to spool out the DNA clumps and place them on **black paper** for observation.

TODAY - DNA CLEANING KIT



Please take the protocol

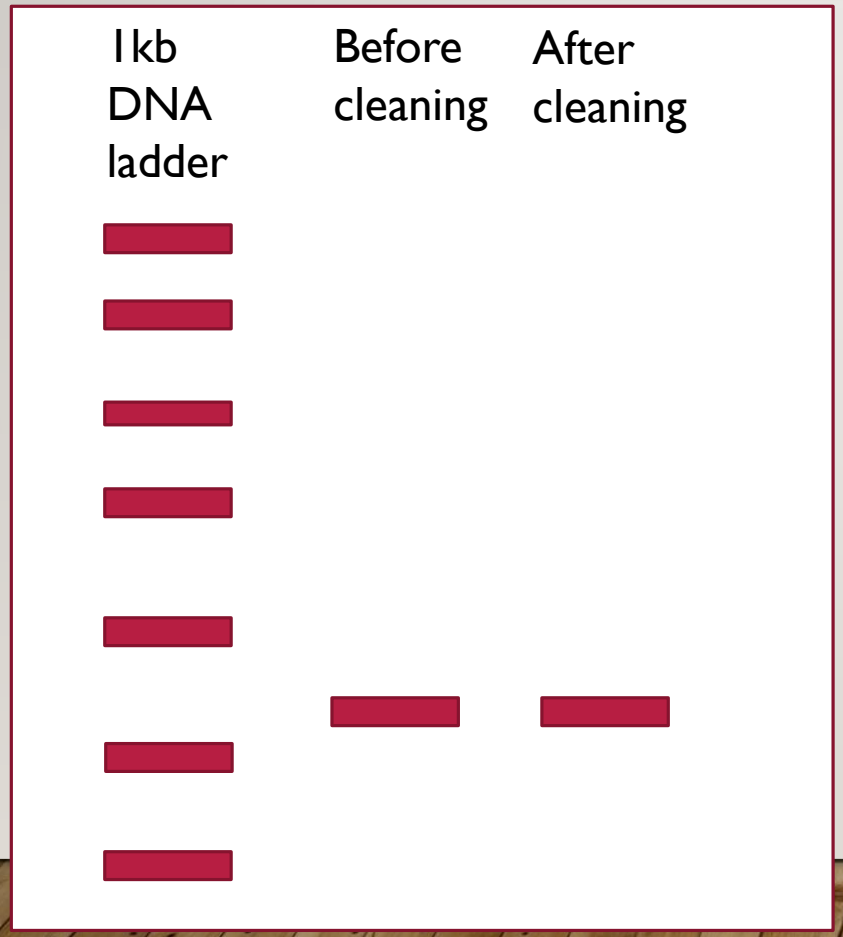
<u>Let's think about the role of each step!</u>

WHAT WE WILL DO TODAY…

- Measure the concentration of PCR product
- Cleaning the PCR product (using one tube)
- Making agarose gel (with cyber safe)
- Confirm the cleaned PCR product by gel electrophoresis
- Enzyme reaction (BamHI first)
- Confirm the enzyme reaction by gel electrophoresis
- 2nd Enzyme reaction (XhoI overnight)

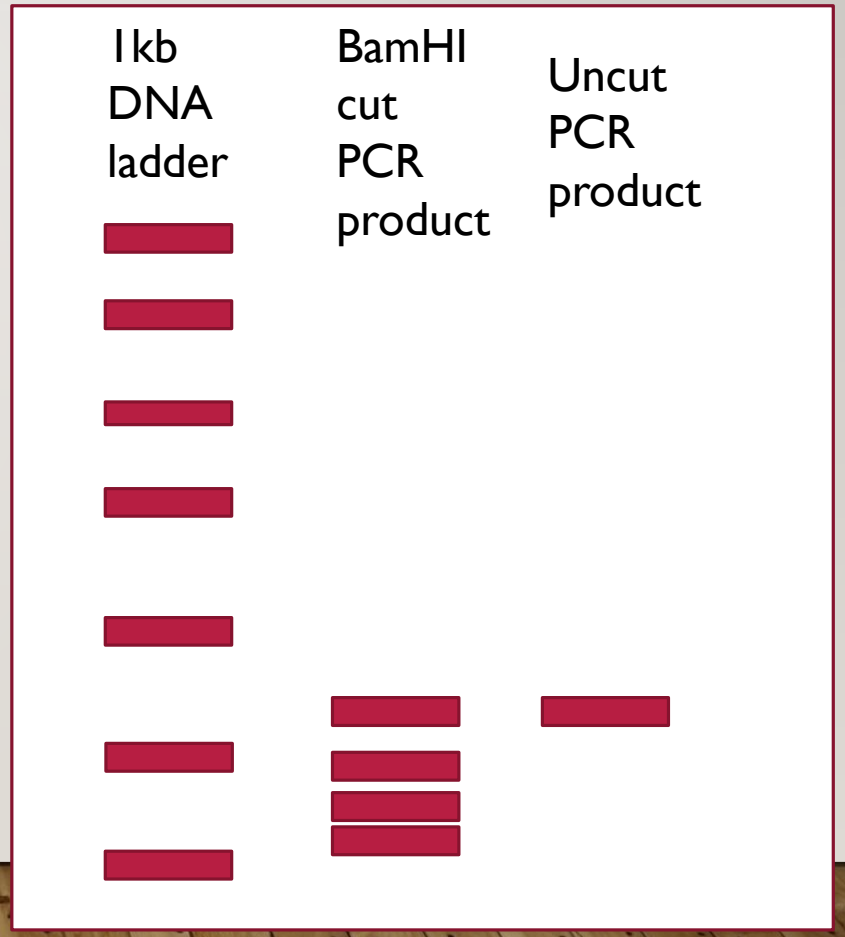
TODAY'S FINAL FIGURE

GOOD LUCK!



Why is it necessary to see the bands?

Think about failed cases!!!



HOMEWORK

- Find what problems can be caused by contamination of DNase and RNase and how to protect from the contamination.
- Explain the glass rod method and mechanism of CsCl method to purify genomic DNA (see p88 the book, gene cloning)
- Find what the ligase is and why this is necessary in real nature.

NEXT WEEK

- Restriction enzyme cutting of template
- BamHI first and then XhoI